MAR 16 2012

510(k) Summary

1. Assigned 510(k) number
   The assigned 510(k) number is **K113420**

2. Company
   Asuragen, Inc.
   2150 Woodward Street, Suite 100
   Austin, TX 78744

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3. Contact
   Luc Van Hove, M.D., Ph.D., CSSBB
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4. Date Prepared
   March 8, 2012

5. Proprietary Name
   Trade name (Proprietary name): RNA*Retain*®

6. Classification Name
   RNA Pre-Analytical System

7. Common Name
   Common name (usual name): RNA*Retain*® collection, storage, and transportation device

8. Classification
   Class II
   Regulation: 21CFR 866.4070
   Product code: OZF
   Panel: Immunology

9. Predicate Device
   K042613, PAXgene® Blood RNA System

10. Device Description – Characteristics

The RNA*Retain*® device consists of an aqueous, hypertonic tissue preservation solution that is provided in a single-use, non-sterile vial intended to serve as the container for the collection, storage, and transport of breast tissue specimens. The ability of the solution formulation to preserve fresh tissue and nucleic acids within the tissue is due to its rapid permeation of tissue to protect cellular nucleic acids from nucleases that would otherwise rapidly degrade the nucleic acids within the specimen. The demonstrated inhibitory effect of the RNA*Retain*® solution on the growth of bacteria, yeast and mold protects the specimen from inadvertent microbial contamination during storage or usage of the device.

The utility of the RNA*Retain*® solution is to maintain high quality cellular RNA for downstream molecular testing. The collection of fresh breast tissue specimens into the RNA*Retain*® device eliminates the need to immediately process these specimens, enabling RNA extraction and molecular testing at a later time and/or transport to a different location. RNA*Retain*® eliminates the need to flash-freeze specimens and to keep specimens frozen throughout storage and transport, a process that is costly and involves manipulation of potentially hazardous agents. It also eliminates the need for formalin fixation, the most common method of clinical tissue preservation, that is both hazardous to work with and known to cross-link and sometimes degrade the nucleic acids rendering them unacceptable for specific downstream molecular applications.

10 a. Device Description – Identification and Materials of Use

The RNA*Retain*® device consists of an aqueous, hypertonic tissue preservation solution that is provided in a single-use, non-sterile vial intended to serve as the container for the collection, storage and transport of breast tissue specimens.

Table 1. RNA*Retain*® configurations.

RNA*Retain*® solution	**Vial Capacity**	**Maximum Tissue Fill**	**Cap**	**Devices per box**	**Comment**
.6 mL	8 mL	0.6 cc or 0.6 g	Leak-proof polyethylene cap	18 vials	Configuration of MammaPrint® includes 1 vial of RNA*Retain*®
5 mL	6 mL	0.5 cc or 0.5 g	Leak-proof polyethylene cap	18 vials	
1 mL	2 mL	0.1 cc or 0.1 g	Leak-proof polypropylene cap with O-ring	24 vials	

10 b. Device Description – Environment of Use

The device will be utilized in the healthcare facility/hospital/laboratory environment.

10 c. Device Description – Key Performance Specifications/Characteristics of the Device

- A single-use, prefilled container intended for fresh tissue collection, storage, and transportation.
- The RNA*Retain*® solution is a non-toxic tissue preservation reagent that stabilizes cellular nucleic acids.
- Eliminates the need to freeze tissue samples after harvesting.
- The preserved tissue is applicable for subsequent nucleic acid isolation and downstream molecular tests.
- The performance characteristics of RNA*Retain*® have been established for RNA with fresh breast tumor tissue and the MammaPrint® Test (k070675) and the additional Asuragen testing.
- Multiple vial configurations containing RNA*Retain*® solution: 6 mL solution in a 8 mL vial; 5 mL solution in a 6 mL vial; 1 mL solution in a 2 mL vial.
- Specimen content of 6 mL: ≤ 0.6 cc of solid tissue; Specimen content of 5 mL: ≤ 0.5 cc of solid tissue; Specimen content of 1 mL: ≤ 0.1 cc of solid tissue. This is a specimen to solution ratio of 1:10 or less.
- Device storage and stability: 5 mL and 1 mL configurations up to 36 months at room temperature (18 to 25 °C) and ≤ 85% humidity; 6 mL configuration up to 20 months at room temperature (18 to 25 °C) and ≤ 85% humidity.
- Tissue should be submerged in RNA*Retain*® for at least 12 hours prior to freezing.
- Tissue can be stored in reagent for up to 3 days at 35 to 39 °C, 7 days at 18 to 25 °C, 30 days at 2 to 8 °C, or 3 years at -15 to -30 °C before RNA extraction.
- Median RNA purity, yield, integrity and corresponding %CVs between assay replicates are equivalent to flash frozen tissue.
- RNA Purity ($A_{260}/A_{280} \geq 1.6$).
- Integrity (28S:18S ≥ 1.0 or RIN ≥ 7.0).
- Ambient temperature storage and shipping of device to end-user.

11. Intended Use/Indications for Use

The RNA*Retain*® device is a single-use, prefilled container intended for the collection, storage, and transportation of fresh breast tissue specimens for subsequent RNA isolation and further molecular diagnostic testing. For Professional Use Only.

11 a. Intended Use – general description of diseases and conditions

The device itself does not diagnose, treat, prevent, cure, or mitigate any disease or condition, but rather is intended for the collection, storage, and transportation of fresh breast tissue.

The difference in tissue type and vial closures from the predicate does not add additional risk to the use of the device.

12. Table of Similarities and Differences of RNA*Retain*® and Predicate device

Table 2. RNA*Retain*®and PAXgene blood system comparison table.

Item	Test Article	Predicate
Name	RNA*Retain*®	PAXgene® Blood RNA System
510(k) number	k113420	k082150; k042613
510(k) holder	Asuragen, Inc. Austin, TX	PreAnalytiX GMBH Feldbachstrasse, Hombrechtikon Switzerland CH-8634
Product Code	OZF	NTW
Intended Use	Pre-analytical specimen collection, storage and transportation device	**SAME**
	RNA*Retain*® is used to preserve fresh breast tissue and stabilize cellular RNA	**SAME**, but tissue type is blood
	The preserved tissue can be used for subsequent nucleic acid isolation and further molecular testing	**SAME,** but system also contains the nucleic acid purification kit for isolation of RNA
	RNA is used for molecular testing	**SAME**
Technology	Deactivation of ribonucleases and preservation of RNA molecules	**SAME**
Principle of Operation	Tissue collection, preservation, storage and transportation	**SAME**, but also includes the isolation of RNA
Components	Single-use, specimen container prefilled with preservation solution	**SAME,** but the system also contains a blood RNA kit for the isolation of RNA
Risks	Chemical exposure risk to person who handles the material-minimum	**SAME**
	RNA degradation risk to patient for misdiagnosis, delay in diagnosis and sample recollection	**SAME**

Item	Test Article	Predicate
Sample types	Breast	**DIFFERENT,** Tissue type is blood
Storage Solution Composition	Ammonium sulfate, sodium citrate and EDTA at specific, proprietary concentrations	**DIFFERENT**, formulated for blood tissue, but same concept - tetradecyltrimethyl-ammonium oxalate and tartaric acid
Sterility	Non-sterile	**DIFFERENT**, Sterile
Container	Transparent, polypropylene plastic tube	**DIFFERENT,** Blood collection tube of undeclared plastic
Closure	Leak-proof polyethylene cap for 6 mL in 8 mL vial and for 5 mL in 6 mL vial	**DIFFERENT,** Special blood collection enclosure.
	Polypropylene cap with O-ring (for 2 mL vial)	

13. Non-Clinical Analytical Performance

Analytical performance Agendia

13 a. Precision/Reproducibility (k070675)
Reproducibility of multiple isolations starting from breast tissue sample collected in RNA*Retain*®.

In order to determine the reproducibility of the MammaPrint® device process from tissue processing to the end result, five previously analyzed tumor samples (one borderline, two high risk and two low risk) were isolated in duplicate. Over multiple days, the ten isolations from five tumors were processed according to standard MammaPrint® protocols.

Table 3. Reproducibility

Sample	Original index	Result	Index from first isolation	Index from second isolation
S1	0.376	High risk (borderline)	0.254	0.374
S2	0.608	Low risk	0.564	0.553
S3	0.659	Low risk	0.639	0.680
S4	-0.105	High risk	0.068	0.067
S5	-0.305	High risk	-0.171	-0.337

No statistically significant difference in MammaPrint® risk group assignment or MammaPrint® index between the two separate RNA isolations was observed.

13 b. Stability of MammaPrint® outcomes in RNARetain® (k070675)
The effect of shipment of a tumor in RNA*Retain*® on stability of MammaPrint® results was demonstrated in k070675. The tumor was selected from which both immediately snap-frozen and RNA*Retain*® preserved sections were available. Both sections of the tumor had a similar tumor cell percentage and similar RNA quality. Both samples were labeled 5 times and hybridized on MammaPrint® microarrays according to standard protocols. MammaPrint® Indices were compared to determine if samples shipped in RNA*Retain*® have a greater stability than tumor sections which are immediately stored at -70 °C after excision. Results showed that the incorporation of both Cy5 and Cy3 were significantly higher for samples shipped in RNA*Retain*® (Unpaired T-tests, p=0.018 and p=0.001 respectively). The variance in MammaPrint® indices was smaller for samples that were stored in RNA*Retain*® compared to the samples that were frozen immediately (Std dev 0.022 vs. 0.042). An unpaired T-test of the MammaPrint® index revealed no significant difference in the actual MammaPrint® indices for the RNA*Retain*® and frozen samples (p=0.24). Based on these experiments, the stability in MammaPrint® index is greater in samples stored in RNA*Retain*® than in samples that were immediately frozen. The difference in MammaPrint® index between RNA*Retain*® and frozen tissue (Δ 0.027) was within the previously determined acceptable limit of index variation.

13 c. Comparison studies
Method comparison with predicate device (k070675):
The samples for this study were collected in 2003 as a pilot study for the Dutch Raster clinical trial sponsored by the Dutch Health Insurance Council where tissue would be shipped in RNA*Retain*® from 20 hospitals. One set consisted of 33 breast tumor samples of which one part of the sample was immediately snap-frozen in liquid nitrogen and stored at -70 °C, another part was stored in RNA*Retain*® for 3 to 5 days at room temperature and subsequently removed from the preservation solution, snap frozen and stored at -70 °C. Another set comprised of 18 tumors of which two parts were available for research that were immediately snap frozen and stored at -70 °C. RNA isolation and DNAse treatment were performed in this same period. HE stained sections were re-examined by a pathologist to confirm invasive ductal carcinoma and sufficient tumor cell content. All samples were hybridized on MammaPrint® microarrays, and passed all sample, labeling and hybridization QCs. Analysis was performed using Feature Extraction version 8.5 and XPrint version 1.40.
Results of MammaPrint® indices of paired RNA*Retain*® and frozen samples are shown in Figure 2A. The median difference between the RNA*Retain*® preserved and the snap frozen is 0.070. The Pearson correlation (0.94) and regression analysis indicate a high similarity ($R^2 = 0.90$).

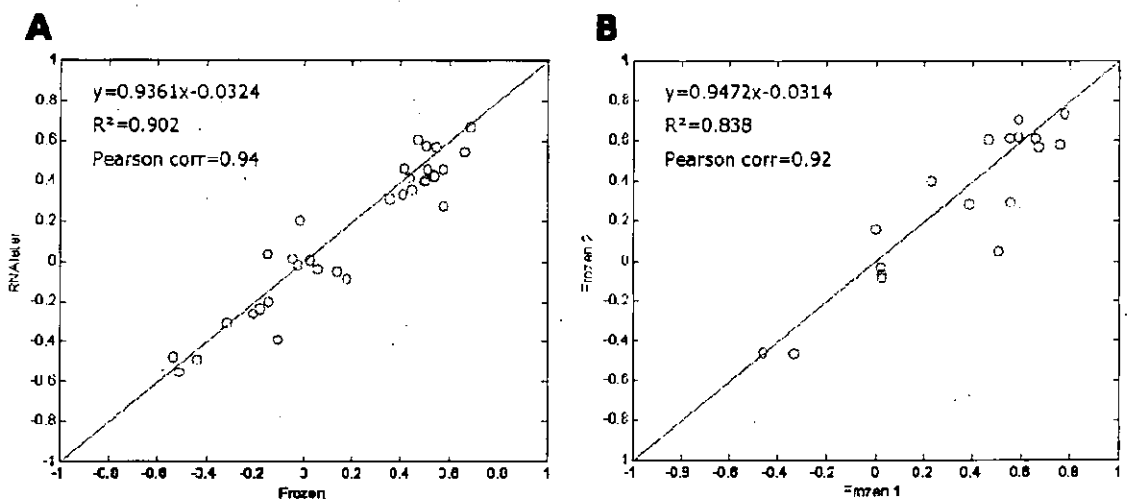

Figure 2: Comparison of MammaPrint® Indices between two samplings of the same tumor. A RNARetain®-frozen; B frozen-frozen.

This finding is similar to the results of a series of tumors of which two frozen samples were available and were collected in the same time period (Figure 2B). The median difference in MammaPrint® Index was 0.105. A comparison of the differences in both series (RNA*Retain*®-frozen vs. frozen-frozen) showed no significant difference *(t-test, p=0.57)* indicating no variation is introduced by RNA*Retain*®.

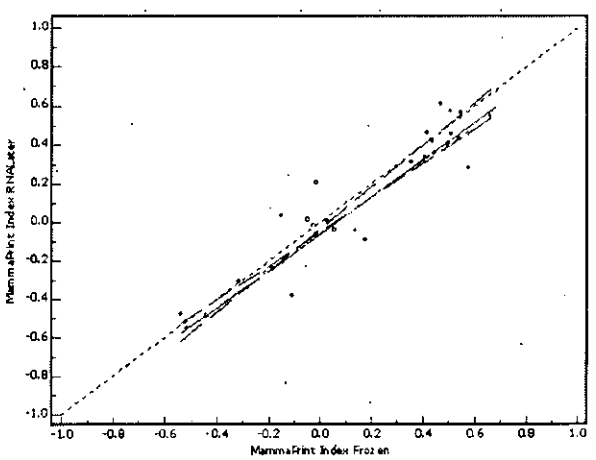

Figure 3: A Passing and Bablok regression analysis on the RNARetain® vs. frozen samples.

Analytical Performance Asuragen:

13 d. Repeatability and Reproducibility

Ten adjacent pairs of breast tissue were collected by Asuragen from 3 subjects. From each pair, one was stored in RNA*Retain*® at 2 to 8 °C for 12 to 18 hours and the other was frozen in liquid nitrogen and stored at ≤ -70 °C. The RNA*Retain*® and flash-frozen stored specimens were then shipped overnight on blue ice or dry ice, respectively. The samples were processed immediately upon receipt for RNA purification and RNA analysis. The average yield, integrity, and purity values for each subject (10 replicates), together with their associated standard deviation (SD) are presented in Table 4. There was no significant difference between tissues from various storage methods, indicating that RNA quality from human breast tissue stored in RNA*Retain*® is equivalent to flash-frozen specimens and that the two RNA preservation methods are compatible with downstream molecular analyses.

Table 4. Mean results for the 3 breast tissue replicates.

		Integrity (28S:18S)		**Purity (A_{260}/A_{280})**		**Yield**
Subject	**Storage**	**AVG**	**SD**	**AVG**	**SD**	**AVG**
1	RNA*Retain*	1.5	0.3	1.97	0.03	148
	Frozen	1.6	0.2	1.92	0.06	131
2	RNA*Retain*	1.6	0.2	2.03	0.04	62
	Frozen	1.5	0.2	2.01	0.11	43
3	RNA*Retain*	1.5	0.2	1.96	0.14	101
	Frozen	1.5	0.1	1.96	0.09	81

13 e. Sample stability

Human breast cancer cell line MCF-7 were grown and resuspended in RNA*Retain*®, incubated overnight at 2-8 °C, then subjected to up to 7 days at 35 to 39 °C, up to 15 days at 18 to 25 °C, up to 60 days at 2 to 8 °C, and up to 3 years at -15 to -30 °C. RNA Yield (A260), Purity (A_{260}/A_{280}), and Integrity (28S:18S ratios) were compared to Fresh Frozen cells. Samples passed acceptance criteria up to 3 days at 35 to 39 °C, 15 days at 18 to 25 °C, 60 days at 2 to 8 °C, and up to 3 years at -15 to -30 °C.

13 f. RNARetain® reagent stability

Stability studies were performed with 3 lots of the 8mL vial (6 mL volume) up to 20 months with storage at room temperature (18 to 25 °C). Reagents passed acceptance criteria. Additional stability studies with the 6 mL vial (5 mL volume) and 2 mL vial (1 mL volume) configurations were also performed up to 36 months and passed acceptance criteria. RNA*Retain*® is stable at room temperature up to 36 months.

Summary of clinical studies for the determination of substantial equivalence

Not Applicable.

Summary of Substantial Equivalence to Predicate

The studies above demonstrate that RNA*Retain*® is safe and effective in the collection, storage and transport of fresh breast tissue; preserving the nucleic acids to allow for high quality RNA isolation and accurate clinical outcome test results with a cleared assay. RNA*Retain*® was compared to the fresh-frozen method for tissue transportation and RNA preservation and determined to be safe, effective, and equivalent to its performance in the Agendia MammaPrint® (k070675) submission.

RNA*Retain*® is substantially equivalent to the predicate - PAXgene® Blood RNA System. With the exception of the RNA isolation kit (which is not a component of the RNA*Retain*® product), the two devices have the same technology, and principle of operation – that is the collection, preservation, storage and transportation of a specific tissue. Both devices are single-use specimen plastic containers prefilled with preservation solution. The risks are the same, which is risk to the patient for misdiagnosis, delay in diagnosis, or sample recollection due to RNA degradation. As the two devices are specific for different tissues (breast vs. blood), a direct comparison between the two devices was not performed; rather each device was compared to the pre-amendment test method utilized for that specific tissue. RNA*Retain*® was compared to the fresh-frozen method of tissue preservation, transportation and storage and PAXgene® was compared to k2EDTA collected blood in an applicable collection vessel. However, the methods used to evaluate the comparison with the pre-amendment test methods were similar: RNA yield (A260) and purity (A260/280). Repeatability and reproducibility evaluations also utilizing RNA yield and purity, and finally a functional evaluation of the RNA quality by molecular diagnostic test assays. Both devices met their acceptance criteria demonstrating these two devices can preserve RNA molecules to yield high quality RNA for subsequent RNA isolation and further molecular diagnostic testing.

The differences in the devices are primarily due to the specific tissue type the device is intended for. PAXgene® has a vial closure that is conducive to drawing blood and the collection vial is sterile as this is a critical parameter for blood which coats the vial during collection. RNA*Retain*® has a vial closure that is closer in composition to the vial and can be sealed after the breast tissue is placed in the vial. The vial is non-sterile as the tissue does not coat the vial during collection and the device solution has been shown to protect against microbial growth in anti-microbial challenge studies. The differences in the devices do not impact their similar intended use, technology, or principle of operation.

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Asuragen, Inc.
c/o Luc Van Hove, M.D., Ph.D., CSSBB
Senior Director Medical/Clinical, Regulatory & Quality
2150 Woodward Street, Suite 100
Austin, TX 78744

MAR 16 2012

Re: k113420
Trade/Device Name: RNA*Retain*®
Regulation Number: 21 CFR §866.4070
Regulation Name: RNA Preanalytical Systems
Regulatory Class: Class II
Product Code: OZF
Dated: January 19, 2012
Received: January 20, 2012

Dear Dr. Van Hove:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing

your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Maria M. Chan

Maria M. Chan, Ph.D.
Director
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): k113420

Device Name: RNA*Retain*®

Indications For Use:

The RNA*Retain*® device is a single-use, prefilled container intended for the collection, storage, and transportation of fresh breast tissue specimens for subsequent RNA isolation and further molecular diagnostic testing.

For Professional Use Only.

Prescription Use ___X__ (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use __N.A.__ (21 CFR 807 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Maria M Chan

Division Sign-Off

Office of In Vitro Diagnostic
Device Evaluation and Safety

510(k) K113420